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(71) Applicants *(for all designated States except US)*: WHITEHEAD INSTITUTE FOR BIOMEDICAL RESEARCH [US/US]; Nine Cambridge Center, Cambridge, MA 02142 (US). THE NETHERLANDS CANCER INSTITUTE [NL/NL]; Plesmanlaan 21, NL-1066 CX Amsterdam (NL).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: KIM, Peter, S. [US/US]; 48 Baskin Road, Lexington, MA (US). SCHUMACHER, Antonius, N., M. [NL/NL]; Kenaupark 22, NL-2011 MT Haarlem (NL).

(74) Agents: GRANAHAN, Patricia et al.; Hamilton, Brook, Smith & Reynolds, P.C., Two Militia Drive, Lexington, MA 02173 (US).

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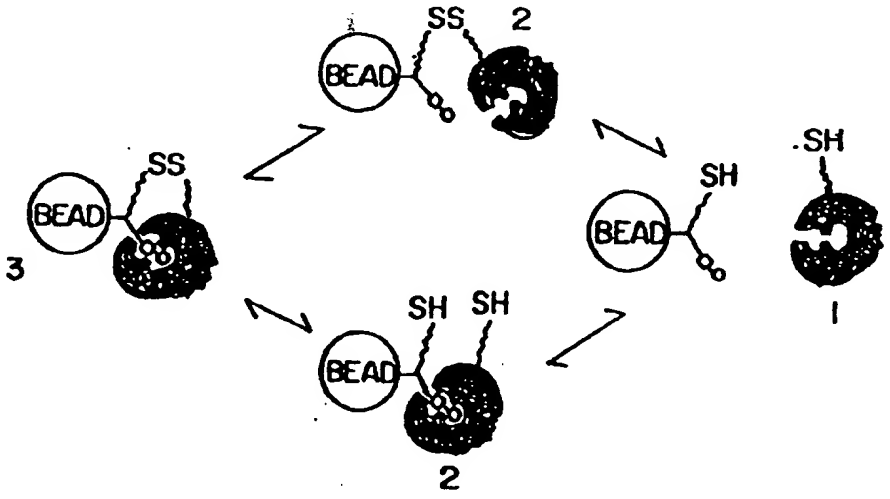
(57) Abstract

Non-specific affinity enhancement as a method of identifying and detecting members, such as ligands and catalysts, in a collection or library of potential ligands or catalysts, which improves the detection limits of such collections or libraries.



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NON-SPECIFIC AFFINITY ENHANCEMENT TO IDENTIFY COMBINATORIAL LIBRARY MEMBERS

Background of the Invention

Combinatorial chemistry is a tool that aids drug discovery efforts. Many different strategies for synthesizing chemicals in a combinatorial manner are available, as are many different strategies for detecting and/or identifying ligands from a combinatorial chemistry library. Unfortunately, available strategies for identifying ligands are unable to detect ligands that do not have a high affinity for the target macromolecule, which is typically a protein. Available strategies for identifying catalysts are similarly limited. As a result, combinatorial chemistry has been most useful when prior knowledge of the nature of ligands is available, so that biased combinatorial chemistry strategies can be utilized. In addition, because the strategies presently available for detecting ligands are unable to detect ligands that do not have a high affinity for a target macromolecule, combinatorial chemistry has been of limited use in identifying chemicals that bind macromolecules of pharmaceutical interest, such as chemicals that can themselves be used for therapeutic or diagnostic purposes or that can serve as lead compounds to be modified to produce a therapeutically effective agent or diagnostic reagent. It would facilitate identification of ligands and catalysts of all types, including those of pharmaceutical interest, if improved methods of detecting ligands of low affinity and catalysts were available.

Summary of the Invention

The present invention relates to a method of identifying a molecule, present in a collection or library, which binds a target molecule or which is a catalyst which acts upon a target molecule. That is, the present invention relates to a method of identifying a ligand for a target molecule and to a method of identifying a catalyst for which the target molecule is a substrate. In the present method, members of a collection or library are linked or tethered in a non-specific manner to or interact in a non-specific manner with target molecules, resulting in improved detection limits of collections or libraries (increasing the likelihood that a ligand or catalyst present in the collection or library will be identified or detected), relative to detection limits using previously-available methods. The subject invention also relates to methods of enhancing the effective concentration of a ligand for a target molecule and of enhancing the effective concentration of a catalyst which acts upon the target molecule, as well as to ligands and catalysts identified by the claimed methods. It further relates to collections or libraries of molecules (potential ligands or potential catalysts) which, as produced or as modified, contain a reactive moiety which is one member of a binding pair. Such libraries are useful in the present methods.

In one embodiment, the present invention relates to a method of identifying and/or detecting a ligand, in a collection or library of potential ligands, of a target molecule, in which potential ligands have the capacity to be linked or tethered in a non-specific manner to target molecules. The potential ligands can be linked or tethered to target molecules reversibly or irreversibly, and can be linked or tethered covalently or non-covalently. As a result, the detection limits of collections or libraries are improved, relative to detection limits using presently

available methods. The present invention also relates to methods of enhancing the effective concentration of a ligand for a target molecule and to ligands identified by the claimed methods.

One embodiment of this invention is a method of identifying or detecting a ligand for a target molecule in a library or collection of potential ligands. The method comprises creating or producing a library or collection of potential ligands which contain a reactive moiety or modifying an existing collection or library of potential ligands to contain a reactive moiety; combining the library or collection with a target molecule which contains, as obtained or as modified, a binding partner for the reactive moiety contained on the potential ligands, to produce a combination; maintaining the combination under conditions appropriate for binding of the reactive moiety and the binding partner, to produce target molecules having tethered thereto a potential ligand, and for specific binding of a target molecule with a ligand; and determining whether specific binding of a target molecule and a potential ligand tethered to the target molecule occurs. If specific binding has occurred, the potential ligand specifically bound to the target molecule is a ligand for the target molecule. Optionally, the complex of the ligand specifically bound to the target molecule can be separated or removed from the library or collection, using known methods. The ligand can also be separated from the ligand-target molecule complex, using known methods.

The binding partner and reactive moiety can be selected from a wide variety of binding pairs/partners, including free sulfhydryl groups and sulfur moieties which are available for disulfide bond formation through exchange; biotin and streptavidin/avidin; leucine zipper components; peptide-binding domains and peptides; ion chelating motifs and ions; ester bonds and other covalent

interactions; aptamers specific for caffeine and caffeine; aptamers specific for ATP and ATP; FK506 and an FK506 binding protein (FKBP); cyclosporin and cyclophilin; steroids and their respective steroid receptors; hormones and their respective hormone receptors; pharmaceutical targets and pharmaceuticals; cyclodextrins and their corresponding binding partners; antibodies and their corresponding antigens; molecules which contain, or are linked to, a magnetic force and a corresponding molecule which is attracted to it; molecules which contain, or are linked to, an electric charge, and a corresponding molecule or substance which is attracted to it; and components of charge-charge interactions. Formation of an interaction between a binding partner and a reactive moiety can be dependent on (controlled by) "cofactors", such as the formation of crosslinks between NH_2 groups that are mediated by succinimide derivatives. The interaction between a binding partner and a reactive moiety can be covalent or non-covalent.

As discussed in detail below, the potential ligands present in a collection or library, as well as the target molecule, can be any of a wide variety of molecules, including proteins (including polypeptides and peptides), oligonucleotides, DNA, RNA, protein nucleic acids, lipoproteins, glycoproteins, carbohydrates, lipids, small organic molecules, phage, viruses, toxins, drugs, membrane proteins, nucleoprotein complexes, pharmaceuticals, hormones, phosphoinositides, prostaglandins, prostacyclins, thromboxanes and large organic molecules. As is also discussed below in detail, a wide variety of linkers can be used.

The present invention permits detection or identification of ligands in collections or libraries at detection limits not possible using previously-available methods. The method, thus, can be used to identify ligands

which would not be available through use of presently-available methods. Ligands identified by the subject method have a wide variety of uses, including as drugs and reagents for therapeutic and diagnostic purposes and as lead molecules for drug design.

In one embodiment of the present invention, the effective concentration of a ligand for a target molecule is enhanced. The target molecule (a molecule for which a ligand is sought) contains, as obtained or as modified, a moiety which is one member of a binding pair. The effective concentration of a ligand for the target molecule is enhanced by creating or producing, using known methods, a collection or library of potential ligands of the target molecule, in which potential ligands contain a reactive moiety which binds the member of the binding pair contained on the target molecule. That is, the reactive moiety is one member of the binding pair whose other member is contained on the target molecule. Alternatively, an existing collection or library of potential ligands can be modified to enhance the effective concentration of a ligand for a target molecule. This is done, using known methods, by modifying library or collection components to contain a reactive moiety which binds a binding partner contained on the target molecule. Library or collection components are modified to contain one member of the binding pair; the target molecule, as obtained or as modified, contains the other (second) member of the binding pair.

A second embodiment of this invention is a method of identifying or detecting, in a library or collection of potential catalysts, a catalyst of a reaction in which the target molecule is a substrate. The method comprises creating or producing a library or collection of potential catalysts which contain a reactive moiety or modifying an existing collection or library of potential catalysts to contain a reactive moiety; combining the library or

collection with a target molecule which contains, as obtained or modified, a binding partner for the reactive moiety contained on the potential catalysts, to produce a combination; maintaining the combination under conditions appropriate for binding of the reactive moiety and the binding partner, to produce target molecules having tethered thereto a potential catalyst and for a potential catalyst to act upon the target molecule and carry out a chemical transformation; and determining whether a potential catalyst tethered to a target molecule catalyzes a reaction in which the target molecule is acted upon. If catalysis has occurred (if the target molecule is acted upon), the target molecule is a substrate of the potential catalyst (the potential catalyst is a catalyst). Alternatively, it is possible to identify a substrate for a known catalyst by screening a library of potential substrates with a target molecule which is a catalyst. The present method of identifying substrates permits detection or identification of substrates in collections or libraries at detection limits which are improved relative to detection limits using previously-known methods. In this embodiment, the linker used to tether potential catalysts to target molecules must be inert to (must not be a substrate of) the catalyst identified. Linking or tethering of a potential catalyst and a target molecule can be irreversible or reversible, and covalent or non-covalent, in this embodiment.

In this embodiment as well, the binding partner and reactive moiety can be selected from a wide variety of binding pairs/partners, including free sulfhydryl groups and sulfur moieties which are available for disulfide bond formation through exchange; biotin and streptavidin/avidin; leucine zipper components; peptide-binding domains and peptides; ion chelating motifs and ions; aptamers specific for caffeine and caffeine; aptamers specific for ATP and

ATP; FK506 and an FK506 binding protein (FKBP); cyclosporin and cyclophilin; steroids and their respective steroid receptors; hormones and their respective hormone receptors; pharmaceutical targets and pharmaceuticals; cyclodextrins and their corresponding binding partners; antibodies and their corresponding antigens; molecules which contain, or are linked to, a magnetic force and a corresponding molecule which is attracted to it; molecules which contain, or are linked to, an electric charge and a corresponding molecule or substance which is attracted to it and components of charge-charge interactions.

The potential catalysts in a collection or library, as well as the target molecule, can be any of a wide variety of molecules, including proteins (including polypeptides and peptides), oligonucleotides, DNA, RNA, protein nucleic acids, lipoproteins, glycoproteins, carbohydrates, lipids, small organic molecules, phage, viruses, toxins, drugs, membrane proteins, nucleoprotein complexes, pharmaceuticals, hormones, phosphoinositides, prostaglandins, prostacyclins, thromboxanes and large organic molecules.

The present method of identifying catalysts permits detection or identification of catalysts in collections or libraries at detection limits not possible using presently-available methods, thus making it possible to identify catalysts with lower catalytic activity than is presently possible. Such catalysts can be used, as obtained, or can be modified to produce catalysts with altered (e.g., greater or longer-lived) activity. The catalysts can be used in research and in commercial or industrial settings, such as to produce pharmaceuticals, materials such as plastics and other polymers, and other products, such as food products, detergents and other cleansers and oral hygiene products (e.g., toothpastes, mouthwashes).

Brief Description of the Drawings

Figure 1 is a schematic representation of one embodiment of the method of the present invention, in which the potential ligands are present on beads.

Figure 2 is a schematic representation of one embodiment of the method of the present invention, in which the target molecule is attached to a cell membrane.

Figure 3 is a graphic representation of results of HPLC analysis of retention time of various reaction products resulting from analysis of the effect of a second, non-specific interaction on the identification of ligands for targets of interest.

Figure 4 is a graphic representation which shows that Crk-binding peptide TS-8 preferentially forms disulfide-bonded heterodimers with the Crk SH3 under native conditions, but not under denaturing conditions.

Detailed Description of the Invention

The present invention relates to a method of improving identification or detection of a molecule, in a library or collection of molecules, which is a binding partner for a target molecule or acts catalytically upon a target molecule (which is a substrate of the molecule identified). Thus, the present invention is a method of identifying or detecting a ligand which binds a target molecule or a catalyst which acts upon a target molecule. A particular advantage of the embodiment of the present method by which ligands are identified or detected is that no prior knowledge of the ligands or of the structure of a target molecule, including the binding site, is needed. Similarly, a particular advantage of the embodiment by which catalysts are identified or detected is that it is not necessary to know anything about the catalysts or the target molecule (substrate) structure.

Identification or Detection of Ligands

In one embodiment, the present invention relates to a method of improving identification of a ligand for a target molecule, in which the ligand is present in a collection or library of potential ligands. The method is generally applicable, due to the fact that the affinity of ligands for their target molecule(s) is enhanced in a non-specific manner. As a result, ligand(s) for any selected target molecule can be identified and/or detected even though the affinity of the ligand, present in a library, for the target molecule is lower than the affinity which is necessary for identification and/or detection using presently available methods.

In the present method, a potential ligand for a target molecule is linked or tethered, in a non-specific manner, to a target molecule, thus increasing the apparent or effective concentration of the ligand for the target molecule. The non-specific binding of a potential ligand to a target molecule is effected by binding of a reactive moiety present on the potential ligand with a binding partner on the target molecule. The ligand can be linked or tethered to the target molecule covalently or non-covalently, and reversibly or irreversibly. In the embodiment in which linking is reversible, the likelihood that the ligand and target molecule will bind and remain bound under the conditions used is increased. For non-specific binding pairs for which a formed interaction is irreversible, the conditions used can be chosen such that the chance of such an interaction occurring are small in the absence of a specific interaction (e.g., by manipulation of pH, addition of untethered reactive moiety, etc.). Because a specific interaction increases the effective concentration of the two members of the binding pair, the chance of formation of an irreversible complex is increased and the formation of such complexes can be detected. An irreversible, non-specific binding pair can

also be utilized in situations where the chance of a specific interaction occurring is small in the absence of a non-specific binding interaction, provided that detection of the occurrence of a specific interaction is possible. As a result of the present invention, the detection limits of combinatorial libraries and other collections of molecules are improved (relative to detection limits using presently-available methods), for example, by 100-fold or more. Detection at millimolar affinity levels of the ligand is easily accomplished using the present invention, even in cases where there is only a single specific interaction site on the target molecule for the ligand.

Thermodynamics requires that the change or difference in ligand affinity (when tethered and untethered ligands are compared) is directly related to the free energy change for the first interaction between a potential ligand and the target molecule. For example, following the initial reaction (for example, non-specific binding of a ligand with a target molecule through the binding of members of a binding pair), each subsequent reaction (in this case, specific binding of the ligand to the target molecule to which it is tethered) is more favorable because the entropy loss is less (as compared to the entropy loss in the absence of the tether). The non-specific interaction is chosen to act in a favorable manner for binding of the ligand and its target molecule. Alternatively, if the initial reaction is specific binding of a ligand with the target molecule, the subsequent reaction (binding of the members of the binding pair) is more favorable.

For the method of the present invention to be carried out, a potential ligand and the target molecule must each contain, as obtained or as modified, a member of a binding pair, in order to permit non-specific linking or tethering of a potential ligand with the target molecule. The member of the binding pair present on a potential ligand is

referred to here as a reactive moiety or first member of a binding pair; the member present on the target molecule is referred to as a binding partner of the reactive moiety or second member of the binding pair.

Libraries of potential ligands can be produced, using known methods, in which members contain (as produced) a reactive moiety. For example, phage display libraries which comprise a single cysteine residue followed by a random sequence of amino acids can be used. As obtained, a target molecule might also include a binding partner (such as a sulfur moiety within a cysteine residue) which is available or can be made available (e.g., as a free sulfhydryl group or sulfur that is available for disulfide bond formation through exchange) for binding with a reactive moiety. If such a target molecule is used, potential ligands can be modified to include a free sulfhydryl group or a sulfur that is available for disulfide bond formation through exchange, which can be attached to the potential ligands via a linker or can be present on (attached directly to) the potential ligands. Here, non-specific binding of target molecule and potential ligands occurs through formation of a disulfide bond. Alternatively, potential ligands and target molecules can be modified to contain, respectively, a reactive moiety and a binding partner, using known methods.

In the present method of enhancing the effective concentration of a ligand for a target molecule, the target molecule contains, as it is obtained or as modified, a binding partner which is one member of a binding pair and is a binding partner for a reactive moiety present on members of a library of potential ligands for the target molecule. In the method, the target molecule containing the binding partner is combined with a library of potential ligands, in which the potential ligands contain the reactive moiety which is the second member of the binding

pair. A method of enhancing the effective concentration of a ligand for a target molecule which contains a binding molecule which is one member of a binding pair comprises modifying potential ligands in a library in such a manner that they contain the reactive moiety which is the second member of the binding pair.

In one embodiment of the present invention, the effective concentration of a ligand for a target molecule is enhanced in the following manner: the ligand is modified by the addition of a covalent, flexible linker and a reactive moiety, in such a manner that the linker is positioned between the ligand and the reactive moiety (in this embodiment, the order is ligand - linker - reactive moiety). The linker can be attached to the ligand at any point on the ligand (e.g., at either end or at an internal position). As obtained or as modified, the target molecule contains a binding partner for the reactive moiety which is attached to the ligand. As a result, the ligand and target molecule are non-specifically linked or tethered when the reactive moiety and its binding partner bind and the effective concentration of the ligand is enhanced. The effective concentration of the ligand for the target molecule is the concentration of untethered ligand that would be required, in order to obtain the same likelihood for binding between the (untethered) ligand and the target molecule, as the likelihood that the tethered ligand and target molecule will bind. Similarly, the effective concentration of the reactive moiety for the binding partner is the concentration of untethered reactive moiety required to obtain the same likelihood for binding the binding partner, as the likelihood that a tethered reactive moiety and a binding partner will bind. Because effective concentration is enhanced through use of the present method, ligands which would otherwise not be identified or detected bind the target molecule.

In one embodiment of the present method of identifying a ligand for a target molecule, a library of potential ligands is provided. In this embodiment, wherein each potential ligand has attached thereto a linker and a reactive moiety, the library can be one which, as created or produced, comprises potential ligands containing a reactive moiety or can be an existing library in which members have been modified to contain reactive moieties. The library of potential ligands is combined with a target molecule which contains a binding partner for the reactive moiety attached to the potential ligands, thereby producing a combination. The combination is maintained under conditions appropriate for binding of the reactive moiety and the binding partner and for specific binding of a target molecule with a potential ligand. The interactions which occur in the combination are represented schematically in Figure 1, in which, for illustrative purposes only, potential ligands are shown attached to beads. Initially (step 1), potential ligands and target molecules are present in the combination as separate entities. Subsequently (step 2), two interactions occur: binding of the reactive moiety on potential ligands with the binding partner on target molecules (referred to as non-specific binding) and binding of ligand(s) with the target molecule (referred to as specific binding). These interactions may occur individually (e.g., only one type of interaction occurs), sequentially (e.g., one type of interaction occurs, followed by the second type) or simultaneously. Whether the first interaction to occur between a potential ligand and a target molecule is nonspecific binding or specific binding, the result is that the effective concentration of the potential ligand (and, thus, the likelihood a ligand will bind a target molecule and remain bound) is increased. Potential ligands which bind specifically to target molecules are ligands of the

target molecule; potential ligands which do not bind target molecules are not ligands of the target molecules. The result of these interactions is a mixture, which may include any or all of the following: untethered potential ligands, untethered target molecules, tethered potential ligands, untethered ligands and tethered ligands. Specific binding of a target molecule and a potential ligand tethered thereto is determined (and, thus, a ligand of the target molecule is identified), using known methods. Once a ligand has been identified, it can be characterized. A ligand can also be separated from the complex it has formed with the target molecule. If further ligands are desired (e.g., with greater binding affinity), knowledge of the characteristics of the ligand can be used to design a biased library of potential ligands (e.g., a library of potential ligands in which a region of the ligand identified which appears to be critical for binding is varied based on characterization of the ligand identified).

The linker used in relation to the potential ligands and target molecules described herein serves the purpose of colocalization of a reactive moiety and a potential ligand and of a binding partner and a target molecule. It is only necessary that the linker be sufficiently flexible and of sufficient length to permit simultaneous specific and non-specific binding (and maintenance of bonds formed) under the conditions used. As a result, the linker can be any of a wide variety of types, including a covalent bond, a single component or unit (e.g., one amino acid), a chain of units (e.g., amino acids) joined together, a solid surface (e.g., beads or planar surfaces), and a cell membrane or cell membrane fragment. The linker can be, for example, simply a bond (e.g., a covalent bond) introduced between a component of a potential ligand and a reactive group added to the potential ligand. Alternatively, the linker can be comprised of one or more amino acids (e.g., 6-aminocaproic

acid), other organic units or a combination thereof. The amino acids can be naturally occurring (modified or unmodified) or nonnaturally occurring.

The target molecule can be attached to or present within a cell membrane (or cell membrane fragment), which serves to colocalize it with the binding partner. In this embodiment, the membrane serves as a linker. For example, a cell surface receptor for which drugs which bind are sought can be the target molecule; the receptor is present on a cell surface. (See Figure 2) If the cell used also expresses a second moiety which is a binding partner, such as FKBP, (which is attached to the cell membrane via a membrane anchor), the target molecule and binding partner (FKBP) are linked by means of the membrane. Cells or cell fragments containing both can be contacted with a collection or library of potential ligands which bear a reactive moiety which binds the binding partner on the cell membrane (here, FK-506). For example, as shown in Figure 2, the library can be comprised of potential ligands joined to FK-506 by means of a linker. Recognition and binding of FK-506 with the FKBP on the cell membrane tethers the potential ligand with the target molecule, increasing the effective concentration relative to one another and enhancing the likelihood they will bind and a ligand will be identified. Alternatively, potential ligands can be present on or attached to cell membranes or fragments, which also bear a reactive moiety, and the target molecule (bearing a binding partner) can be contacted with the cells.

The target molecule can also be contacted with potential ligands in an embodiment in which the target molecule (or molecules) is present on a chip, which also bears a binding partner, such as free sulfhydryl groups. Here, the linker between target molecule and binding partner is the chip. For example, a protein or a

collection of proteins for which a ligand(s) is sought can be used. Contacting the chip with a library of potential ligands which bear a reactive moiety results in identification of ligands, if present, in the library. Alternatively, a library of potential ligands, each of which contains a reactive moiety, can be contained on a chip, which is contacted with a target molecule containing a binding partner.

It is not necessary that both potential ligands (or catalysts) and target molecules contain a linker or if both do contain a linker, that the two linkers are of the same length or composition. It is only necessary that potential ligands and target molecules contain, respectively, a reactive moiety and a binding partner of the reactive moiety and that these two members of a binding pair are able to interact to link or tether a potential ligand to a target molecule in such a manner that the two remain joined and can interact with one another under the conditions used.

In a specific embodiment, the covalent, flexible linker is a synthetic peptide or polypeptide. The linker is a peptide which comprises a sufficient number of amino acid residues to be long enough to provide suitable space for the ligand to simultaneously interact with and bind the target molecule in a specific and non-specific manner. Attached to the peptide is a reactive group or moiety which is available to bind with a binding partner present on a target molecule. The amino acid residues in the linker can be any of the naturally-occurring amino acids (modified or unmodified), non-naturally occurring amino acids or a combination thereof. The linker can be, for example, $(glycine\text{-}glycine\text{-}serine)_n$-cysteine, where n can equal any number, provided that the linker is not so short that it prevents simultaneous specific and non-specific interaction and binding of a ligand and a target molecule. In this

embodiment, the cysteine provides a free sulfhydryl group, which is available to react with a free sulfhydryl group present on the target molecule (as obtained or as modified). The interaction of the two sulfhydryl groups results in tethering of the ligand to the target molecule and thermodynamically more favorable conditions for binding of the ligand with the target molecule. The non-specific interaction (here, of the disulfide groups) acts in a favorable manner for binding and, as a result, ligands that could not be identified in the absence of the non-specific interaction can be identified. In this embodiment, the likelihood of non-specific interaction (here, disulfide bond formation) can be varied by adjusting the concentration of external oxidizing and reducing agents (for example, oxidized and reduced glutathione, respectively) present in the solution. The direct thermodynamic relationship also provides an alternative strategy for identifying ligands from a combinatorial library; molecules that bind with higher affinity will necessarily increase the effective concentration of the other members of the binding pair to a greater extent. Thus, in this embodiment, tethered ligands that bind with higher affinity will have disulfide bonds that are more resistant to reduction by external reducing agents, such as reduced glutathione.

The library of potential ligands to be screened can be any collection or library of molecules of interest, provided that the molecules as produced contain or can be appropriately modified to contain a reactive moiety. For example, the collection or library can be a chemical library, a combinatorial chemistry library, a combinatorial biologically-encoded library (e.g., a SELEX library or a phage display library), a collection of protein variants (e.g., produced by random mutagenesis of a gene encoding the protein or produced by site-directed mutagenesis of

codons for selected residues in the protein), a cell lysate, cell culture medium, a fungal broth, a library obtained through simultaneous coupling of mixtures of building blocks, or a library formed through spatially addressable synthesis. As discussed above, the library can be present in solution or on a solid surface. If the library is on a solid surface (e.g., beads, plane), or on a cell membrane, one or more potential ligands can be present on a surface. For example, one potential ligand can be displayed per bead or a mixture or two or more potential ligands can be attached to a bead. The library of potential ligands can be an unbiased or biased library. A biased library is one which is limited or restricted to a particular set of entities or molecules and is not completely random. It includes a high frequency of molecules that contain chemical entities or building blocks which are known or thought to interact with a molecule for which a ligand is sought or contains chemical entities which resemble the structure and/or chemical properties of chemical entities known or thought to interact with a molecule for which a ligand is sought.

The nonspecific interaction between potential ligands and target molecules can occur through interactions between reactive moieties and their binding partners other than sulfhydryl groups. For example, the interactions can be those that occur between biotin and streptavidin/avidin, leucine zipper components, peptide-binding domains and peptides (e.g., SH3 domains), ion chelating motifs and ions (e.g., EF hands, His tags), DNA binding domains (e.g., zinc fingers), ester bonds and other covalent interactions, aptamers that are specific for compounds such as caffeine or ATP, and, respectively, caffeine or ATP, antibodies and their corresponding antigens, pharmaceuticals (e.g., FK506, cyclosporin), with known ligands (e.g., FKBP, cyclophilin), steroids and their respective steroid receptors, hormones

and their respective receptors, antibodies and their corresponding antigens, electric fields and charge-charge interactions. The non-specific interactions between potential ligands and target molecules can be covalent or non-covalent.

The likelihood of the non-specific interaction can be varied by use of an untethered reactive moiety or untethered binding partner. For example, in an embodiment in which the non-specific interaction occurs between biotin (tethered to the ligand) and avidin (tethered to the target molecule), the likelihood of non-specific interaction can be decreased by adding increasing concentrations of either untethered biotin and/or untethered avidin. The likelihood of non-specific interaction in this embodiment can also be decreased by dilution: at lower concentration of potential ligands and/or target molecules, the likelihood of interaction will decrease. In an embodiment in which the non-specific interaction occurs, through ester bond formation, the likelihood of non-specific interaction can be varied by altering the pH of the solution.

In one embodiment, a library of molecules (referred to as a library of potential ligands) which are to be screened for their ability to bind a target molecule is produced. Each member of the library comprises a variable region (which is the potential ligand), a linker (also referred to as a spacer) and a reactive moiety. The library of potential ligands can be attached to a solid surface (e.g., beads, plates), using known techniques, or can be maintained in solution.

In one embodiment, beads for synthesis of potential ligands are first modified to introduce a low level of sulfhydryl groups. A library that consists of potential ligands is then synthesized on these beads, using the "one-bead, one-compound" strategy. Here, the bead itself is the linker. The library is then incubated, under oxidizing

conditions, with a version of the target macromolecule containing a natural or artificial sulfhydryl group, so that a disulfide bond is formed between the target and the sulfhydryl on the bead. The beads are then washed for variable periods of time, under conditions of varying concentrations of reducing agent, followed by incubation in the presence of a sulfhydryl quenching agent, such as iodoacetate. The beads may then be washed under denaturing conditions to remove any non-covalently bound target. Beads that still contain bound target can be detected readily (for example, by antibody binding detection methods).

Target molecules for which ligands can be identified using the present method include proteins (including polypeptides and peptides), oligonucleotides, DNA, RNA, protein nucleic acids, lipoproteins, glycoproteins, carbohydrates, lipids, small organic molecules, phage, viruses, toxins, drugs, membrane proteins, nucleoprotein complexes, pharmaceuticals, hormones, phosphoinositides, prostaglandins, prostacyclins, thromboxanes and large organic molecules. A target molecule, as obtained, contains or is modified to contain a binding partner for the reactive moiety present on the potential ligands in the library to be screened. For example, in the case in which the reactive moiety present on potential ligands is a free sulfhydryl group, the target molecule will also contain or be modified to contain a free sulfhydryl group. Optionally, the target molecule can also be modified to contain a detectable moiety, useful to detect the presence of the ligand-target molecule complex formed. For example, the target molecule can be modified to include a (biotin)-(biotinylation signal). Modification of the target molecule can be an amino terminal modification, a carboxyl-terminal modification or an internal modification (modification of a non-terminal amino acid residue) if the

target molecule is a peptide, polypeptide or protein (all referred to herein as proteins). Similarly, if the target is a molecule of another type (i.e., not a protein), the modification can be a terminal (end) or internal modification.

Once specific binding of a ligand in a library of potential ligands with the target molecule has occurred, detection of the resulting complex (ligand bound to target molecule) can be carried out using known methods. Interaction of ligand and target molecule can be detected by, for example, using a target molecule which contains or is modified with an antibody epitope (e.g., SH3) containing a domain which can be identified by an antibody and/or staining or sorting with anti-tag antibodies.

The methods described herein permit the detection and identification of ligands for target molecules (such as physiologically significant mammalian proteins, biological molecules involved in diseases, small organic molecules, pharmaceuticals, toxins, proteins and oligonucleotides critical for activity of infectious organisms), particularly ligands which are not detected or identified by previously-available methods because they do not have a sufficiently high affinity for the target molecule. Ligands identified by this method are useful, for example, as drug candidates, which can be assessed, using known methods, for their ability to alter activity of the target molecule and, if necessary, can be modified to enhance their therapeutic effects. Such ligands can also be used in diagnostic methods, such as a method of diagnosing the presence of or monitoring the status of a disease with which the target molecule is associated.

Identification of Catalysts

In a further embodiment, a catalyst for (a molecule which acts catalytically upon) a target molecule is identified. In this embodiment, a random population

(collection or library) of molecules is assessed or searched for the presence of a member for which the target molecule is a catalytic substrate. The subject method makes it possible to identify molecules with weak (low) catalytic activity, which are not identifiable by previously-available methods. Here, as in the embodiment which results in identification of a ligand, members of a collection or library are tethered non-specifically to a target molecule. The linkage can be reversible or non-reversible, and covalent or non-covalent. Potential catalysts in the library or collection, as obtained or modified, bear a reactive moiety and the target molecule, as obtained or modified, bears a binding partner for the reactive moiety.

The binding partner and reactive moiety can be selected from a wide variety of binding pairs/partners, including free sulfhydryl groups and sulfur moieties which are available for disulfide bond formation through exchange; biotin and streptavidin/avidin; leucine zipper components; peptide-binding domains and peptides; ion chelating motifs and ions; ester bonds and other covalent interactions; aptamers specific for caffeine and caffeine; aptamers specific for ATP and ATP; FK506 and an FK506 binding protein (FKBP); cyclosporin and cyclophilin; steroids and their respective steroid receptors; hormones and their respective hormone receptors; pharmaceutical targets and pharmaceuticals; cyclodextrins and their corresponding binding partners; antibodies and their corresponding antigens; molecules which contain, or are linked to, a magnetic force and a corresponding molecule which is attracted to it; molecules which contain, or are linked to, an electric charge, and a corresponding molecule or substance which is attracted to it; and components of charge-charge interactions.

The potential catalysts present in a collection or library, as well as the target molecule for which a catalyst is sought can also be any of a wide variety of molecules, including proteins (including polypeptides and peptides), oligonucleotides, DNA, RNA, protein nucleic acids, lipoproteins, glycoproteins, carbohydrates, lipids, small organic molecules, phage, viruses, toxins, drugs, membrane proteins, nucleoprotein complexes, pharmaceuticals, hormones, phosphoinositides, prostaglandins, prostacyclins, thromboxanes and large organic molecules.

The library of potential catalysts used can be any collection or library of molecules, provided the molecules contain, as obtained or modified, a reactive moiety which binds a binding partner present on the target molecule used. For example, the collection or library can be a chemical library, a combinatorial chemistry library, a combinatorial biologically-encoded library (e.g., a SELEX library or a phage display library), a collection of protein variants (e.g., produced by random mutagenesis of a gene encoding the protein or produced by site-directed mutagenesis of codons for selected residues in the protein), a cell lysate, cell culture medium, a fungal broth, a library obtained through simultaneous coupling of mixtures of building blocks, or a library formed through spatially addressable synthesis. As discussed above, the library can be present in solution or on a solid surface. If the library is on a solid surface (e.g., beads, plane), or on a cell membrane, one or more potential catalysts can be present on a surface. For example, one potential catalyst can be displayed per bead or a mixture of two or more potential catalysts can be attached to a bead.

When the library of potential catalysts which contain the reactive moiety is combined with the target molecule which contains the binding partner and the resulting

combination is maintained under appropriate conditions (for binding of the reactive moiety and the binding partner and for a catalytic reaction to occur in which the target molecule is acted upon and a chemical transformation occurs), potential catalysts become tethered to the target molecule, thus enhancing the effective concentration of a catalyst in the library and the target molecule (substrate). Catalytic activity, thus, is more likely to occur than is the case with presently available methods.

Catalytic activity (which indicates that a catalyst has been identified) can be assessed or shown by a variety of methods. For example, if a catalyst for a reaction which results in cleavage of a target molecule which is a protein (e.g., angiotensin) is desired, the target protein can be labelled by addition of a fluorescent group (fluorophore) at one location (e.g., at one end) and addition of a fluorescence quencher at another location (e.g., at the other end). For example, a bead or other solid surface can be used as the linker and the linking carried out such that the fluorophore remains attached to the target protein after cleavage of the protein occurs (e.g., into two fragments). Cleavage of the protein in a reaction catalyzed by a member of the library results in removal of the peptide fragment which bears the fluorescence quencher. Therefore, although the beads bearing the labelled target protein were initially non-fluorescent (due to the presence of the quencher), they become fluorescent if a catalyst is present in the library or collection (as a result of the catalyst's acting upon the target protein).

In another embodiment, the library assessed can be a library of protein variants, such as a library of variants of a protein, in which the variants retain the ability to fold in a manner similar to that of the wild type (nonvariant or "parent") protein. For example, the library

can be a collection of variants of GB1 (a small 56 - residue protein which includes loops), which contain a combinatorial assortment of amino acid residues in the loops of the protein. The substrate in this embodiment is a peptide to be cleaved. As above, the peptide is labelled with a fluorophore and a fluorescence quencher, typically one at each end of the peptide. The linker, which is a bead or other solid surface, is attached to the peptide such that after cleavage occurs, the fluorophore remains attached to the peptide fragment that remains attached to the bead or solid surface, but the quencher has been removed. That cleavage has occurred (and, thus, the presence of a catalyst) is easily demonstrated by fluorescence in the combination produced when the library and target molecule are combined and maintained under conditions appropriate for linking of potential catalysts with the target peptide and cleavage of the peptide. Fluorescence is shown by shining a light of appropriate wavelength on the combination. The GB1 variants on fluorescent beads can be sequenced to determine which members of the library are catalytically active.

Other detection methods can also be used, such as colorimetric and antibody - based methods.

The present invention is illustrated by the following examples, which are not intended to be limiting in any way.

EXAMPLE 1: Isolation of Ligands for the Crk N-SH3 Domain Through Non-specific Affinity Enhancement

The following is a description of identification of ligands in a collection or library in which ligands are covalently attached to a solid surface (beads) during synthesis.

The NH_2-terminal SH3 domain of the c-Crk protein is a 58 amino acid domain that lacks cystein residues. The 3-

dimensional structure of a complex of this domain with a peptide ligand has been solved by X-ray crystallography. To identify novel ligands for the NH_2-terminal SH3 domain of the c-Crk protein, using non-specific affinity enhancement the following experiment may be performed:

A). Derivatives of the NH_2-terminal Crk SH3 domain with a COOH-terminal linker and free sulfhydryl, and an NH_2-terminal biotin/biotinylation signal are generated by conventional molecular biological and protein expression methods.

(1): (biotin)-(biotinylation signal)-(c-Crk residue 134-191)-$(Gly\text{-}Gly\text{-}Ser)_3$-Cys

(2): (biotin)-(biotinylation signal)-(c-Crk residue 134-191)-$(Gly\text{-}Gly\text{-}Ser)_4$-Cys

Synthetic linkers with the sequence $(Gly\text{-}Gly\text{-}Ser)_3$-Cys and $(Gly\text{-}Gly\text{-}Ser)_4$-Cys are produced by solid phase peptide synthesis.

B). Beads for solid phase peptide synthesis are modified by coupling of a substochiometric amount of sidechain-protected N-acetylated cysteine (e.g., 1:5 ratio of cysteine to reactive groups). A library of synthetic compounds is subsequently produced on these beads using cycles of split and pool synthesis, using FMOC-protected monomer building blocks as described (K.S. Lam et al., Nature, 1991; A.P. Combs et al., JACS, 1996).

C). The biotinylated SH3 domain derivatives and the corresponding synthetic linkers (SH3:linker;1:10) are incubated with the library of compounds, in Tris buffer (10 mM, pH 7.5), in the presence of a redox system (e.g., reduced glutathione (GSH) and oxidized glutathione (GSSG) at various ratios). The inclusion of an excess of the corresponding (unbiotinylated) synthetic linkers is optional and is used to select against compounds that interact with the linker, rather than with the SH3 domain. The beads are subsequently washed with buffer under

increasingly reducing conditions. The reaction is subsequently quenched by the addition of acetic acid (final concentration of 5%), and the beads are washed with a denaturing buffer (6 M Guanidine-HCl/5% HAc) to remove any non-covalently bound SH3 domain. The beads are then washed with 10 mM Tris pH 7.5/100 mM iodoacetamide and incubated with streptavidin-alkaline phosphatase. The presence of bound SH3 domain is subsequently determined by visual inspection of the beads under a light microscope. Beads that are positive are collected and the identity of the compounds on these beads is determined by microsequencing.

EXAMPLE 2: Demonstration of the Effect of a Non-Specific Interaction on the Identification of Ligands for Targets of Interest

To demonstrate the effect if a second non-specific interaction on the identification of ligands for targets of interest, the following experiment was performed. A derivative of the NH2-terminal c-Crk SH3 domain with a NH2-terminal Ha-tag and a C COOH-terminal (GGS)3 linker, followed by a free cysteine residue was incubated with a derivative of a Crk SH3 ligand with an NH2-terminal linker and free cysteine residue. Disulfide bond formation between the two free cysteine residues in the presence or absence of denaturant was monitored by reverse phase HPLC. The sequences of the two derivatives are, respectively: amino-AYPYDVPDYASAEYVRALFDFNGNDEEDLPFKKGDILRIRDK-PEEQWWNAEDSEGKRGMIPVPYVEKYRGGSGGSGGSC-carboxyl and acetyl-CGGSGGSPPPALPPKKR-carboxyl.

Figure 3 indicates the retention times of the various reaction products on c-18 reverse phase HPLC. Figure 4 indicates that the Crk-binding peptide TS-8 preferentially forms disulfide-bonded heterodimers with the Crk SH3 domain under native conditions, but not under denaturing

conditions. This experiment illustrates that under conditions where a specific interaction between a target molecule and ligand can take place, preferential formation of disulfide-medicated ligand-target heterodimers can be observed.

Reaction conditions: Peptide and SH3 domain (200 and 50 μM respectively) were incubated under anaerobic conditions for 18-24 hours in 100 mM Tris (pH 8.7)/200 mM KCl/1 mM EDTA/1 mM GSSG (glutathione dimer) in the presence of absence of 8M urea. The reaction was terminated by the addition of 5% HAc (final concentration) and the reaction products were quantitated by reverse phase HPLC on a Waters C-18 column.

EXAMPLE 3: Isolation of Ligands Through Non-covalent Affinity Enhancement

The following is a description of identification of ligands in a collection or library, in which ligands are covalently attached to a solid surface (beads) during synthesis. In this example the second interaction is formed through non-covalent interaction: heterodimerization of the pACID and pBASE leucine zippers.

A). A derivative of the NH3 terminal SH3 domain of c-Crk is generated through conventional molecular biology/protein expression techniques.

(1): (Myc tag) - (c-Crk 134-191) - ((GGS)4) - (pACID) (pACID = AQLEKELQALEKENAQLEWELQALEKELAQ, see O'Shea *et al.*, (1993) *Curr. Biol. 3*:658); (Myc tag = EQKLISEEDL) see Boehringer Mannheim product information, pi1667149-12195 and Evan, G.I. *et al.*, *Mol. Cell. Biol. 5*:3610-3616 (1985)/

B). A library of synthetic compounds is generated for instance using cycles of split and pool synthesis (Lam, Nature 1991), with the following structure.

(2): (randomized stretch) - ((GGS(2) - (pBASE)

(pBASE = AQLKKKLQALKKKNAQLKWKLQALKKKLAQ, see O'Shea *et al.*, *Curr. Biol.* *3*:658 (1993))

C). The library of compounds is incubated with the Crk derivative to reveal compounds that interact with this SH3 domain. In this assay the presence of bound SH3 domain is visualized using alkaline phosphatase labeled anti-myc tag antibody (Boehringer Mannheim) by visual inspection of the beads under a light microscope. Anti-c-myc recognizes the 9E10 epitope (sequence EQKLISEEDL), which was derived from the human c-myc protein. Beads that are positive are collected and microsequenced. The inclusion of an excess of free pBASE or pACID peptide is optional and can be used to increase the stringency of the assay.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

CLAIMS

We claim:

1. A method of enhancing the effective concentration of a ligand for a target molecule, wherein the target molecule, as obtained or as modified, contains one member of a binding pair, comprising creating a collection or library of potential ligands for the target molecule, wherein the library is comprised of potential ligands which contain a reactive moiety which is the second member of the binding pair.

2. The method of Claim 1 wherein the binding partner and the reactive moiety are each a free sulfhydryl group or a sulfur moiety which is available for disulfide bond formation through exchange.

3. The method of Claim 1 wherein the binding partner and the reactive moiety are members of a binding pair selected from the group consisting of:
   a) biotin and streptavidin/avidin;
   b) leucine zipper components;
   c) peptide-binding domains and peptides;
   d) ion chelating motifs and ions;
   e) covalent interactions;
   f) aptamers specific for caffeine and caffeine;
   g) aptamers specific for ATP and ATP;
   h) FK506 and an FK506 binding protein (FKBP);
   I) cyclosporin and cyclophilin;
   j) steroid receptors and steroids;
   k) hormone receptors and hormones;
   l) pharmaceutical targets and pharmaceuticals;
   m) cyclodextrins and their corresponding binding partners;

n) antibodies and their corresponding antigens;
o) molecules which contain, or are linked to, a magnetic force and a corresponding molecule which is attracted to it;
p) molecules which contain, or are linked to, an electric charge and a molecule that is attracted to it; and
q) charge-charge interactions.

4. A method of enhancing the effective concentration of a ligand for a target molecule which, as obtained or as modified, contains a binding partner which is one member of a binding pair and is a binding partner for a reactive moiety in a library of potential ligands for the target molecule, comprising modifying potential ligands in the library in such a manner that they contain the reactive moiety which is the second member of the binding pair.

5. The method of Claim 4 wherein the binding partner and the reactive moiety are each a free sulfhydryl group or a sulfur moiety which is available for disulfide bond formation through exchange.

6. The method of Claim 4 wherein the binding partner and the reactive moiety are members of binding pairs selected from the group consisting of:
a) biotin and streptavidin/avidin;
b) leucine zipper components;
c) peptide-binding domains and peptides;
d) ion chelating motifs and ions;
e) covalent interactions;
f) aptamers specific for caffeine and caffeine;
g) aptamers specific for ATP and ATP;
h) FK506 and an FK506 binding protein (FKBP);

i) cyclosporin and cyclophilin;
j) steroid receptors and steroids;
k) hormone receptors and hormones;
l) pharmaceutical targets and pharmaceuticals;
m) cyclodextrins and their corresponding binding partners;
n) antibodies and their corresponding antigens;
o) molecules which contain, or are linked to, a magnetic force and a corresponding molecule which is attracted to it;
p) molecules which contain, or are linked to, an electric charge and a molecule that is attracted to it; and
q) charge-charge interactions.

7. A method of enhancing the effective concentration of a ligand for a target molecule, wherein the target molecule as obtained or as modified contains a binding partner, comprising modifying the ligand by the addition of a linker and a reactive moiety which binds the binding partner contained on the target molecule, such that the linker is positioned between the ligand and the reactive moiety.

8. The method of Claim 7 wherein the linker is selected from the group consisting of:
a) covalent bonds
b) single units;
c) chains of units;
d) solid surfaces;
e) cell membranes; and
f) cell membrane fragments.

9. The method of Claim 7 wherein the binding partner and the reactive moiety are each a free sulfhydryl group or a sulfur moiety which is available for disulfide bond formation through exchange.

10. The method of Claim 7 wherein the binding partner and the reactive moiety are members of binding pairs selected from the group consisting of:

a) biotin and streptavidin/avidin;
b) leucine zipper components;
c) peptide-binding domains and peptides;
d) ion chelating motifs and ions;
e) covalent interactions;
f) aptamers specific for caffeine and caffeine;
g) aptamers specific for ATP and ATP;
h) FK506 and an FK506 binding protein (FKBP);
i) cyclosporin and cyclophilin;
j) steroid receptors and steroids;
k) hormone receptors and hormones;
l) pharmaceutical targets and pharmaceuticals;
m) cyclodextrins and their corresponding binding partners;
n) antibodies and their corresponding antigens;
o) molecules which contain, or are linked to, a magnetic force and a corresponding molecule which is attracted to it;
p) molecules which contain, or are linked to, an electric charge and a molecule that is attracted to it; and
q) charge-charge interactions.

11. The method of Claim 1 wherein the target molecule is selected from the group consisting of: proteins, oligonucleotides, DNA, RNA, protein nucleic acids, lipoproteins, glycoproteins, carbohydrates, lipids,

small organic molecules, phage, viruses, toxins, drugs, membrane proteins, nucleoprotein complexes, pharmaceuticals, hormones, phosphoinositides, prostaglandins, prostacyclins, thromboxanes and large organic molecules.

12. The method of Claim 4 wherein the target molecule is selected from the group consisting of: proteins, oligonucleotides, DNA, RNA, protein nucleic acids, lipoproteins, glycoproteins, carbohydrates, lipids, small organic molecules, phage, viruses, toxins, drugs, membrane proteins, nucleoprotein complexes, pharmaceuticals, hormones, phosphoinositides, prostaglandins, prostacyclins, thromboxanes and large organic molecules.

13. The method of Claim 7 wherein the target molecule is selected from the group consisting of: proteins, oligonucleotides, DNA, RNA, protein nucleic acids, lipoproteins, glycoproteins, carbohydrates, lipids, small organic molecules, phage, viruses, toxins, drugs, membrane proteins, nucleoprotein complexes, pharmaceuticals, hormones, phosphoinositides, prostaglandins, prostacyclins, thromboxanes and large organic molecules.

14. A method of identifying a ligand for a target molecule, in a library of potential ligands, comprising the steps of:

a) producing a library of potential ligands wherein the potential ligands contain a reactive moiety;

b) combining the library of potential ligands with a target molecule which contains, as obtained or modified, a binding partner for the reactive

moiety contained on the potential ligands, thereby producing a combination;

c) maintaining the combination under conditions appropriate for binding of the reactive moiety and the binding partner to produce target molecules having tethered thereto a potential ligand and for specific binding of a target molecule with a ligand; and

d) determining whether specific binding of a target molecule and a potential ligand tethered thereto occurs, wherein if specific binding has occurred, the potential ligand specifically bound to the target molecule is a ligand for the target molecule.

15. The method of Claim 14 wherein the binding partner and the reactive moiety are each a free sulfhydryl group or a sulfur moiety which is available for disulfide bond formation through exchange.

16. The method of Claim 14 wherein the binding partner and the reactive moiety are members of binding pairs selected from the group consisting of:

a) biotin and streptavidin/avidin;
b) leucine zipper components;
c) peptide-binding domains and peptides;
d) ion chelating motifs and ions;
e) covalent interactions;
f) aptamers specific for caffeine and caffeine;
g) aptamers specific for ATP and ATP; and
h) FK506 and an FK-506 binding partner;
i) cyclosporin and cyclosporin;
j) steroid receptors and steroids;
k) hormone receptors and hormones;
l) pharmaceutical targets and pharmaceuticals;

m) cyclodextrins and their corresponding binding partners;

n) antibodies and their corresponding antigens;

o) molecules which contain, or are linked to, a magnetic force and a corresponding molecule which is attracted to it;

p) molecules which contain, or are linked to, an electric charge and a molecule that is attracted to it; and

q) charge-charge interactions.

17. The method of Claim 14 wherein the target molecule is selected from the group consisting of: proteins, oligonucleotides, DNA, RNA, protein nucleic acids, lipoproteins, glycoproteins, carbohydrates, lipids, small organic molecules, phage, viruses, toxins, drugs, membrane proteins, nucleoprotein complexes, pharmaceuticals, hormones, phosphoinositides, prostaglandins, prostacyclins, thromboxanes and large organic molecules.

18. A method of identifying, in a library of potential ligands, a ligand for a target molecule, comprising the steps of:

a) creating a library of potential ligands, wherein each potential ligand has attached thereto a linker and a reactive moiety, wherein the linker is positioned between the ligand and the reactive moiety;

b) combining the library of potential ligands with a target molecule which contains a binding partner for the reactive moiety attached to the potential ligands, thereby producing a combination;

c) maintaining the combination under conditions appropriate for binding of the reactive moiety

and the binding partner to produce target molecules having tethered thereto a potential ligand and for specific binding of a target molecule with a potential ligand; and

d) determining whether specific binding of a target molecule and a potential ligand occurs, wherein if specific binding has occurred, the potential ligand specifically bound to the target molecule is a ligand for the target molecule.

19. The method of Claim 18 wherein the linker is selected from the group consisting of:

a) covalent bonds
b) single units;
c) chains of units;
d) solid surfaces;
e) cell membranes; and
f) cell membrane fragments.

20. The method of Claim 18 wherein the binding partner and the reactive moiety is each a free sulfhydryl group or a sulfur moiety which is available for disulfide bond formation through exchange.

21. The method of Claim 18 wherein the binding partner and the reactive moiety are members of binding pairs selected from the group consisting of:

a) biotin and streptavidin/avidin;
b) leucine zipper components;
c) peptide-binding domains and peptides;
d) ion chelating motifs and ions;
e) covalent interactions;
f) aptamers specific for caffeine and caffeine;
g) aptamers specific for ATP and ATP; and
h) FK506 and an FK-506 binding partner;

i) cyclosporin and cyclophilin;
j) steroid receptors and steroids;
k) hormone receptors and hormones;
l) pharmaceutical targets and pharmaceuticals;
m) cyclodextrins and their corresponding binding partners;
n) antibodies and their corresponding antigens;
o) molecules which contain, or are linked to, a magnetic force and a corresponding molecule which is attracted to it;
p) molecules which contain, or are linked to, an electric charge and a molecule that is attracted to it; and
q) charge-charge interactions.

22. The method of Claim 18 wherein the target molecule is selected from the group consisting of: proteins, oligonucleotides, DNA, RNA, protein nucleic acids, lipoproteins, carbohydrates, glycoproteins, small organic molecules, pharmaceuticals, hormones, phosphoinositides, prostaglandins, prostacyclins, thrombokinase, membrane proteins, nucleoprotein complexes, phage, viruses, large organic molecules, and toxins.

23. The method of Claim 18 wherein the potential ligands are present on a solid surface.

24. The method of Claim 18 wherein the solid surface is beads.

25. The method of Claim 24 wherein more than one potential ligand is present on each bead.

26. The method of Claim 24 wherein a single potential ligand is present on each bead.

27. The method of Claim 18 wherein the target molecule additionally has attached thereto a detectable moiety.

28. The method of Claim 27 wherein the detectable moiety is a biotin-biotinylation signal or an antibody epitope.

29. The method of Claim 18 wherein the potential ligands are present on a cell membrane.

30. A method of enhancing the effective concentration of a ligand for a target molecule, wherein the target molecule, as obtained or modified, contains one member of a binding pair, comprising modifying the ligand to contain a reactive moiety which is the second member of the binding pair.

31. A method of enhancing the effective concentration of a catalyst for a target molecule, wherein the target molecule, as obtained or as modified, contains one member of a binding pair, comprising creating a collection or library of potential catalysts for the target molecule, wherein the library is comprised of potential catalysts which contain a reactive moiety which is the second member of the binding pair.

32. The method of Claim 31 wherein the binding partner and the reactive moiety are each a free sulfhydryl group or a sulfur moiety which is available for disulfide bond formation through exchange.

33. The method of Claim 31 wherein the binding partner and the reactive moiety are members of a binding pair selected from the group consisting of:

a) biotin and streptavidin/avidin;
b) leucine zipper components;
c) peptide-binding domains and peptides;
d) ion chelating motifs and ions;
e) covalent interactions;
f) aptamers specific for caffeine and caffeine;
g) aptamers specific for ATP and ATP;
h) FK506 and an FK506 binding protein (FKBP);
i) cyclosporin and cyclophilin;
j) steroid receptors and steroids;
k) hormone receptors and hormones;
l) pharmaceutical targets and pharmaceuticals;
m) cyclodextrins and their corresponding binding partners;
n) antibodies and their corresponding antigens;
o) molecules which contain, or are linked to, a magnetic force and a corresponding molecule which is attracted to it;
p) molecules which contain, or are linked to, an electric charge and a molecule that is attracted to it; and
q) charge-charge interactions.

34. A method of enhancing the effective concentration of a catalyst for a target molecule which, as obtained or as modified, contains a binding partner which is one member of a binding pair and is a binding partner for a reactive moiety in a library of potential catalysts for the target molecule, comprising modifying potential catalysts in the library in such a manner that they contain the reactive moiety which is the second member of the binding pair.

35. The method of Claim 34 wherein the binding partner and the reactive moiety are each a free sulfhydryl group or a sulfur moiety which is available for disulfide bond formation through exchange.

36. The method of Claim 34 wherein the binding partner and the reactive moiety are members of binding pairs selected from the group consisting of:

a) biotin and streptavidin/avidin;
b) leucine zipper components;
c) peptide-binding domains and peptides;
d) ion chelating motifs and ions;
e) covalent interactions;
f) aptamers specific for caffeine and caffeine;
g) aptamers specific for ATP and ATP;
h) FK506 and an FK506 binding protein (FKBP);
i) cyclosporin and cyclophilin;
j) steroid receptors and steroids;
k) hormone receptors and hormones;
l) pharmaceutical targets and pharmaceuticals;
m) cyclodextrins and their corresponding binding partners;
n) antibodies and their corresponding antigens;
o) molecules which contain, or are linked to, a magnetic force and a corresponding molecule which is attracted to it;
p) molecules which contain, or are linked to, an electric charge and a molecule that is attracted to it; and
q) charge-charge interactions.

37. A method of enhancing the effective concentration of a catalyst for a target molecule, wherein the target molecule as obtained or as modified contains a binding partner, comprising modifying the catalyst by the

addition of a linker and a reactive moiety which binds the binding partner contained on the target molecule, such that the linker is positioned between the catalyst and the reactive moiety.

38. The method of Claim 37 wherein the linker is selected from the group consisting of:
a) covalent bonds
b) single units;
c) chains of units;
d) solid surfaces;
e) cell membranes; and
f) cell membrane fragments.

39. The method of Claim 37 wherein the binding partner and the reactive moiety are each a free sulfhydryl group or a sulfur moiety which is available for disulfide bond formation through exchange.

40. The method of Claim 37 wherein the binding partner and the reactive moiety are members of binding pairs selected from the group consisting of:
a) biotin and streptavidin/avidin;
b) leucine zipper components;
c) peptide-binding domains and peptides;
d) ion chelating motifs and ions;
e) covalent interactions;
f) aptamers specific for caffeine and caffeine;
g) aptamers specific for ATP and ATP;
h) FK506 and an FK506 binding protein (FKBP);
i) cyclosporin and cyclophilin;
j) steroid receptors and steroids;
k) hormone receptors and hormones;
l) pharmaceutical targets and pharmaceuticals;

m) cyclodextrins and their corresponding binding partners;

n) antibodies and their corresponding antigens;

o) molecules which contain, or are linked to, a magnetic force and a corresponding molecule which is attracted to it;

p) molecules which contain, or are linked to, an electric charge and a molecule that is attracted to it; and

q) charge-charge interactions.

41. The method of Claim 31 wherein the target molecule is selected from the group consisting of: proteins, oligonucleotides, DNA, RNA, protein nucleic acids, lipoproteins, glycoproteins, carbohydrates, lipids, small organic molecules, phage, viruses, toxins, drugs, membrane proteins, nucleoprotein complexes, pharmaceuticals, hormones, phosphoinositides, prostaglandins, prostacyclins, thromboxanes and large organic molecules.

42. The method of Claim 34 wherein the target molecule is selected from the group consisting of: proteins, oligonucleotides, DNA, RNA, protein nucleic acids, lipoproteins, glycoproteins, carbohydrates, lipids, small organic molecules, phage, viruses, toxins, drugs, membrane proteins, nucleoprotein complexes, pharmaceuticals, hormones, phosphoinositides, prostaglandins, prostacyclins, thromboxanes and large organic molecules.

43. The method of Claim 37 wherein the target molecule is selected from the group consisting of: proteins, oligonucleotides, DNA, RNA, protein nucleic acids, lipoproteins, glycoproteins, carbohydrates, lipids,

small organic molecules, phage, viruses, toxins, drugs, membrane proteins, nucleoprotein complexes, pharmaceuticals, hormones, phosphoinositides, prostaglandins, prostacyclins, thromboxanes and large organic molecules.

44. A method of identifying a catalyst for a target molecule, in a library of potential catalysts, comprising the steps of:

a) producing a library of potential catalysts wherein the potential catalysts contain a reactive moiety;

b) combining the library of potential catalysts with a target molecule which contains, as obtained or modified, a binding partner for the reactive moiety contained on the potential catalysts, thereby producing a combination;

c) maintaining the combination under conditions appropriate for binding of the reactive moiety and the binding partner to produce target molecules having tethered thereto a potential catalyst and for a potential catalyst to act upon the target molecule and carry out a chemical transformation; and

d) determining whether a catalytic reaction occurs in which a catalyst acts upon the target molecule and carries out a chemical transformation, wherein if such a catalytic reaction occurs, the potential catalyst is a catalyst for the target molecule.

45. The method of Claim 44 wherein the binding partner and the reactive moiety are each a free sulfhydryl group or a sulfur moiety which is available for disulfide bond formation through exchange.

46. The method of Claim 44 wherein the binding partner and the reactive moiety are members of binding pairs selected from the group consisting of:

a) biotin and streptavidin/avidin;
b) leucine zipper components;
c) peptide-binding domains and peptides;
d) ion chelating motifs and ions;
e) covalent interactions;
f) aptamers specific for caffeine and caffeine;
g) aptamers specific for ATP and ATP;
h) FK506 and an FK-506 binding partner;
i) cyclosporin and cyclosporin;
j) steroid receptors and steroids;
k) hormone receptors and hormones;
l) pharmaceutical targets and pharmaceuticals;
m) cyclodextrins and their corresponding binding partners;
n) antibodies and their corresponding antigens;
o) molecules which contain, or are linked to, a magnetic force and a corresponding molecule which is attracted to it;
p) molecules which contain, or are linked to, an electric charge and a molecule that is attracted to it; and
q) charge-charge interactions.

47. The method of Claim 44 wherein the target molecule is selected from the group consisting of: proteins, oligonucleotides, DNA, RNA, protein nucleic acids, lipoproteins, glycoproteins, carbohydrates, lipids, small organic molecules, phage, viruses, toxins, drugs, membrane proteins, nucleoprotein complexes, pharmaceuticals, hormones, phosphoinositides, prostaglandins, prostacyclins, thromboxanes and large organic molecules.

48. A method of identifying, in a library of potential catalysts, a catalyst for a target molecule, comprising the steps of:

a) creating a library of potential catalysts, wherein each potential catalyst has attached thereto a linker and a reactive moiety, wherein the linker is positioned between the catalyst and the reactive moiety;

b) combining the library of potential catalysts with a target molecule which contains a binding partner for the reactive moiety attached to the potential catalysts, thereby producing a combination;

c) maintaining the combination under conditions appropriate for binding of the reactive moiety and the binding partner to produce target molecules having tethered thereto a potential catalyst and for a potential catalyst to act upon the target molecule and carry out a chemical transformation; and

d) determining whether a catalytic reaction occurs in which a catalyst acts upon the target molecule and carries out a chemical transformation, wherein if such a catalytic reaction occurs, the potential catalyst is a catalyst for the target molecule.

49. The method of Claim 48 wherein the linker is selected from the group consisting of:

a) covalent bonds

b) single units;

c) chains of units;

d) solid surfaces;

e) cell membranes; and

f) cell membrane fragments.

50. The method of Claim 48 wherein the binding partner and the reactive moiety is each a free sulfhydryl group or a sulfur moiety which is available for disulfide bond formation through exchange.

51. The method of Claim 48 wherein the binding partner and the reactive moiety are members of binding pairs selected from the group consisting of:

a) biotin and streptavidin/avidin;
b) leucine zipper components;
c) peptide-binding domains and peptides;
d) ion chelating motifs and ions;
e) covalent interactions;
f) aptamers specific for caffeine and caffeine;
g) aptamers specific for ATP and ATP;
h) FK506 and an FK-506 binding partner;
i) cyclosporin and cyclophilin;
j) steroid receptors and steroids;
k) hormone receptors and hormones;
l) pharmaceutical targets and pharmaceuticals;
m) cyclodextrins and their corresponding binding partners;
n) antibodies and their corresponding antigens;
o) molecules which contain, or are linked to, a magnetic force and a corresponding molecule which is attracted to it;
p) molecules which contain, or are linked to, an electric charge and a molecule that is attracted to it; and
q) charge-charge interactions.

52. The method of Claim 48 wherein the target molecule is selected from the group consisting of: proteins, oligonucleotides, DNA, RNA, protein nucleic acids, lipoproteins, glycoproteins, carbohydrates, lipids,

small organic molecules, phage, viruses, toxins, drugs, membrane proteins, nucleoprotein complexes, pharmaceuticals, hormones, phosphoinositides, prostaglandins, prostacyclins, thromboxanes and large organic molecules.

53. The method of Claim 48 wherein the potential catalysts are present on a solid surface.

54. The method of Claim 48 wherein the solid surface is beads.

55. The method of Claim 54 wherein more than one potential catalyst is present on each bead.

56. The method of Claim 54 wherein a single potential catalyst is present on each bead.

57. The method of Claim 48 wherein the target molecule additionally has attached thereto a detectable moiety.

58. The method of Claim 47 wherein the detectable moiety is a fluorophore.

59. The method of Claim 58 wherein the potential catalysts are present on a cell membrane.

60. A collection or library of potential ligands produced by the method of Claim 1.

61. A collection or library of potential ligands produced by the method of Claim 31.

62. The method of Claim 1 wherein the potential ligand is selected from the group consisting of: proteins, oligonucleotides, DNA, RNA, protein nucleic acids, lipoproteins, glycoproteins, carbohydrates, lipids, small organic molecules, phage, viruses, toxins, drugs, membrane proteins, nucleoprotein complexes, pharmaceuticals, hormones, phosphoinositides, prostaglandins, prostacyclins, thromboxanes and large organic molecules.

63. The method of Claim 4 wherein the potential ligand is selected from the group consisting of: proteins, oligonucleotides, DNA, RNA, protein nucleic acids, lipoproteins, glycoproteins, carbohydrates, lipids, small organic molecules, phage, viruses, toxins, drugs, membrane proteins, nucleoprotein complexes, pharmaceuticals, hormones, phosphoinositides, prostaglandins, prostacyclins, thromboxanes and large organic molecules.

64. The method of Claim 7 wherein the potential ligand is selected from the group consisting of: proteins, oligonucleotides, DNA, RNA, protein nucleic acids, lipoproteins, glycoproteins, carbohydrates, lipids, small organic molecules, phage, viruses, toxins, drugs, membrane proteins, nucleoprotein complexes, pharmaceuticals, hormones, phosphoinositides, prostaglandins, prostacyclins, thromboxanes and large organic molecules.

65. The method of Claim 34 wherein the potential catalyst is selected from the group consisting of: proteins, oligonucleotides, DNA, RNA, protein nucleic acids, lipoproteins, glycoproteins, carbohydrates, lipids, small organic molecules, phage, viruses, toxins,

drugs, membrane proteins, nucleoprotein complexes, pharmaceuticals, hormones, phosphoinositides, prostaglandins, prostacyclins, thromboxanes and large organic molecules.

66. The method of Claim 35 wherein the potential catalyst is selected from the group consisting of: proteins, oligonucleotides, DNA, RNA, protein nucleic acids, lipoproteins, glycoproteins, carbohydrates, lipids, small organic molecules, phage, viruses, toxins, drugs, membrane proteins, nucleoprotein complexes, pharmaceuticals, hormones, phosphoinositides, prostaglandins, prostacyclins, thromboxanes and large organic molecules.

67. The method of Claim 37 wherein the potential catalyst is selected from the group consisting of: proteins, oligonucleotides, DNA, RNA, protein nucleic acids, lipoproteins, glycoproteins, carbohydrates, lipids, small organic molecules, phage, viruses, toxins, drugs, membrane proteins, nucleoprotein complexes, pharmaceuticals, hormones, phosphoinositides, prostaglandins, prostacyclins, thromboxanes and large organic molecules.

68. A library comprising potential ligands for a target molecule, wherein potential ligands each contain a reactive moiety which is one member of a binding pair, the binding pair selected from the group consisting of:

a) biotin and streptavidin/avidin;
b) leucine zipper components;
c) peptide-binding domains and peptides;
d) ion chelating motifs and ions;
e) covalent interactions;

f) aptamers specific for caffeine and caffeine;
g) aptamers specific for ATP and ATP;
h) FK506 and an FK506 binding protein (FKBP);
i) cyclosporin and cyclophilin;
j) steroid receptors and steroids;
k) hormone receptors and hormones;
l) pharmaceutical targets and pharmaceuticals;
m) cyclodextrins and their corresponding binding partners;
n) antibodies and their corresponding antigens;
o) molecules which contain, or are linked to, a magnetic force and a corresponding molecule which is attracted to it;
p) molecules which contain, or are linked to, an electric charge and a molecule that is attracted to it; and
q) charge-charge interactions.

69. A library of Claim 68 wherein the ligands are selected from the group consisting of: proteins (including polypeptides and peptides), oligonucleotides, DNA, RNA, protein nucleic acids, lipoproteins, glycoproteins, carbohydrates, lipids, small organic molecules, phage, viruses, toxins, drugs, membrane proteins, nucleoprotein complexes, pharmaceuticals, hormones, phosphoinositides, prostaglandins, prostacyclins, thromboxanes and large organic molecules.

70. A library comprising potential catalysts of a target molecule, wherein potential catalysts each contain a reactive group which is one member of a binding pair, the binding pair selected from the group consisting of:

a) biotin and streptavidin/avidin;

b) leucine zipper components;
c) peptide-binding domains and peptides;
d) ion chelating motifs and ions;
e) covalent interactions;
f) aptamers specific for caffeine and caffeine;
g) aptamers specific for ATP and ATP;
h) FK506 and an FK506 binding protein (FKBP);
i) cyclosporin and cyclophilin;
j) steroid receptors and steroids;
k) hormone receptors and hormones;
l) pharmaceutical targets and pharmaceuticals;
m) cyclodextrins and their corresponding binding partners;
n) antibodies and their corresponding antigens;
o) molecules which contain, or are linked to, a magnetic force and a corresponding molecule which is attracted to it;
p) molecules which contain, or are linked to, an electric charge and a molecule that is attracted to it; and
q) charge-charge interactions.

71. A library of Claim 70 wherein the catalysts are selected from the group consisting of: proteins, oligonucleotides, DNA, RNA, protein nucleic acids, lipoproteins, glycoproteins, carbohydrates, lipids, small organic molecules, phage, viruses, toxins, drugs, membrane proteins, nucleoprotein complexes, pharmaceuticals, hormones, phosphoinositides, prostaglandins, prostacyclins, thromboxanes and large organic molecules.

72. The method of Claim 23 wherein more than one potential ligand is present on each bead.

73. The method of Claim 29 wherein the potential ligands are present on a cell membrane.

74. The method of Claim 29 wherein one potential ligand is present on a cell membrane.

75. The method of Claim 29 wherein more than one potential ligand is present on a cell membrane.

76. The method of Claim 53 wherein more than one potential catalyst is present on each bead.

77. The method of Claim 53 wherein a single potential catalyst is present on each bead.

78. The method of Claim 59 wherein more than one potential catalyst is present on each bead.

79. The method of Claim 59 wherein a single potential catalyst is present on each bead.

80. The method of Claim 14 wherein the potential ligand is selected from the group consisting of: proteins, oligonucleotides, DNA, RNA, protein nucleic acids, lipoproteins, glycoproteins, carbohydrates, lipids, small organic molecules, phage, viruses, toxins, drugs, membrane proteins, nucleoprotein complexes, pharmaceuticals, hormones, phosphoinositides, prostaglandins, prostacyclins, thromboxanes and large organic molecules.

81. The method of Claim 18 wherein the potential ligand is selected from the group consisting of: proteins, oligonucleotides, DNA, RNA, protein nucleic acids, lipoproteins, glycoproteins, carbohydrates, lipids,

small organic molecules, phage, viruses, toxins, drugs, membrane proteins, nucleoprotein complexes, pharmaceuticals, hormones, phosphoinositides, prostaglandins, prostacyclins, thromboxanes and large organic molecules.

82. The method of Claim 44 wherein the potential catalyst is selected from the group consisting of: proteins, oligonucleotides, DNA, RNA, protein nucleic acids, lipoproteins, glycoproteins, carbohydrates, lipids, small organic molecules, phage, viruses, toxins, drugs, membrane proteins, nucleoprotein complexes, pharmaceuticals, hormones, phosphoinositides, prostaglandins, prostacyclins, thromboxanes and large organic molecules.

83. The method of Claim 48 wherein the potential catalyst is selected from the group consisting of: proteins, oligonucleotides, DNA, RNA, protein nucleic acids, lipoproteins, glycoproteins, carbohydrates, lipids, small organic molecules, phage, viruses, toxins, drugs, membrane proteins, nucleoprotein complexes, pharmaceuticals, hormones, phosphoinositides, prostaglandins, prostacyclins, thromboxanes and large organic molecules.

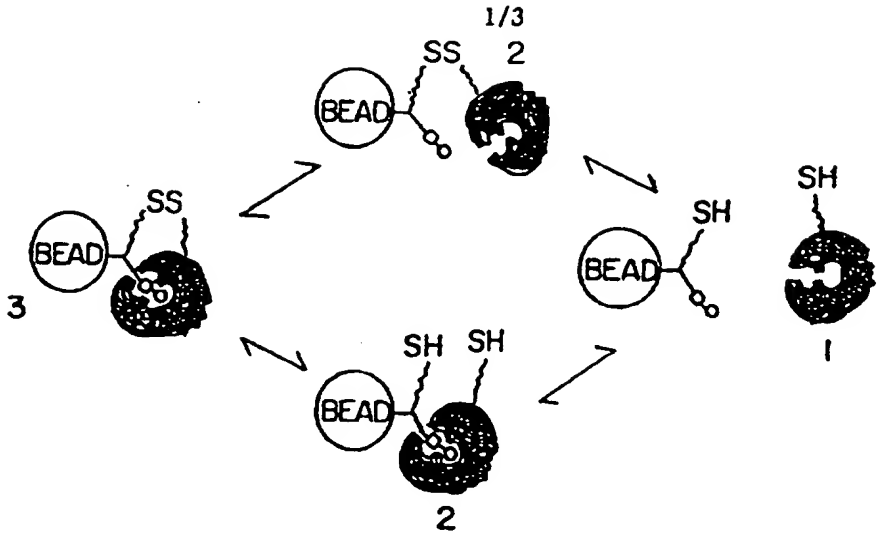
1/3
SS
2
BEAD
SS
BEAD
3
SH
SH
BEAD
1
SH
SH
BEAD
2

FIG. 1

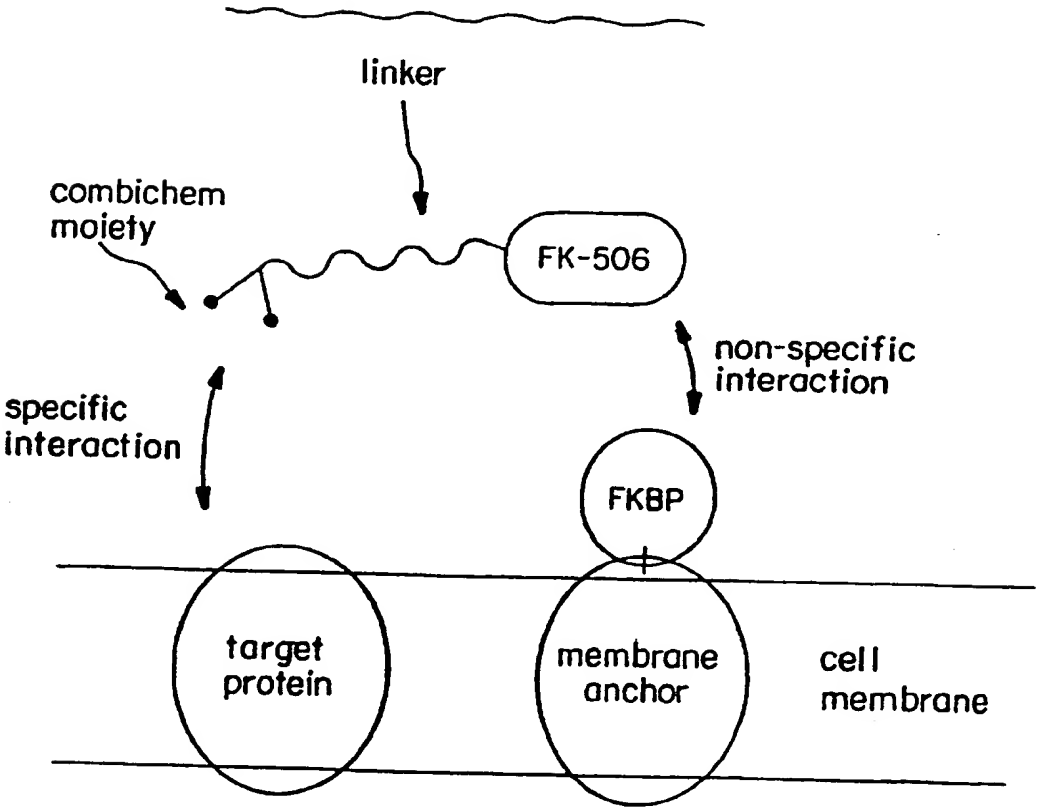
linker
combichem moiety
FK-506
non-specific interaction
specific interaction
FKBP
target protein
membrane anchor
cell membrane

FIG. 2

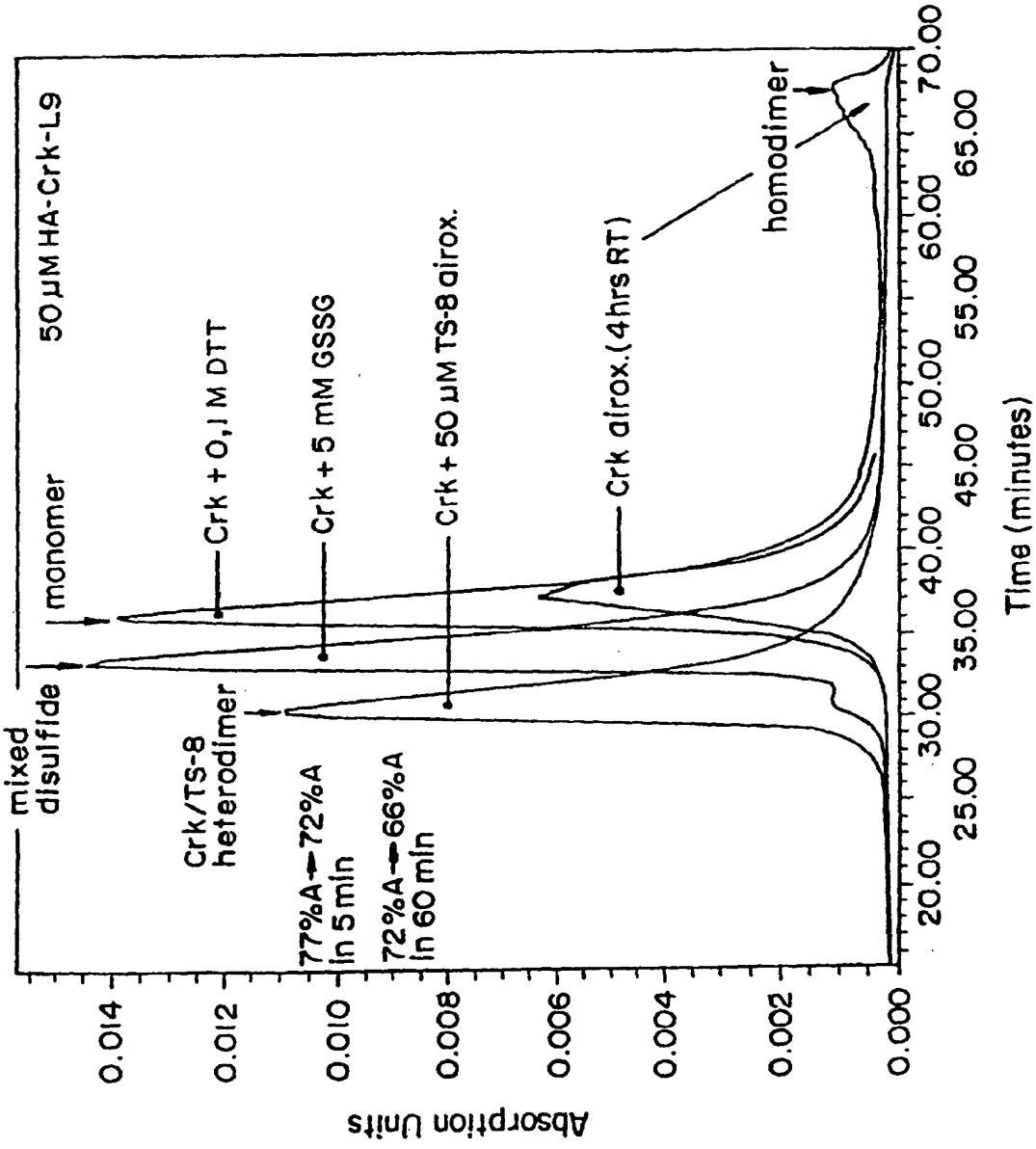
50 µM HA-Crk-L9
monomer
Crk + 0,1 M DTT
Crk + 5 mM GSSG
Crk + 50 µM TS-8 airox.
Crk airox.(4hrs RT)
homodimer
mixed disulfide
Crk/TS-8 heterodimer
77%A→72%A in 5 min
72%A→66%A in 60 min
Absorption Units
0.014
0.012
0.010
0.008
0.006
0.004
0.002
0.000
20.00
25.00
30.00
35.00
40.00
45.00
50.00
55.00
60.00
65.00
70.00
Time (minutes)

FIG. 3

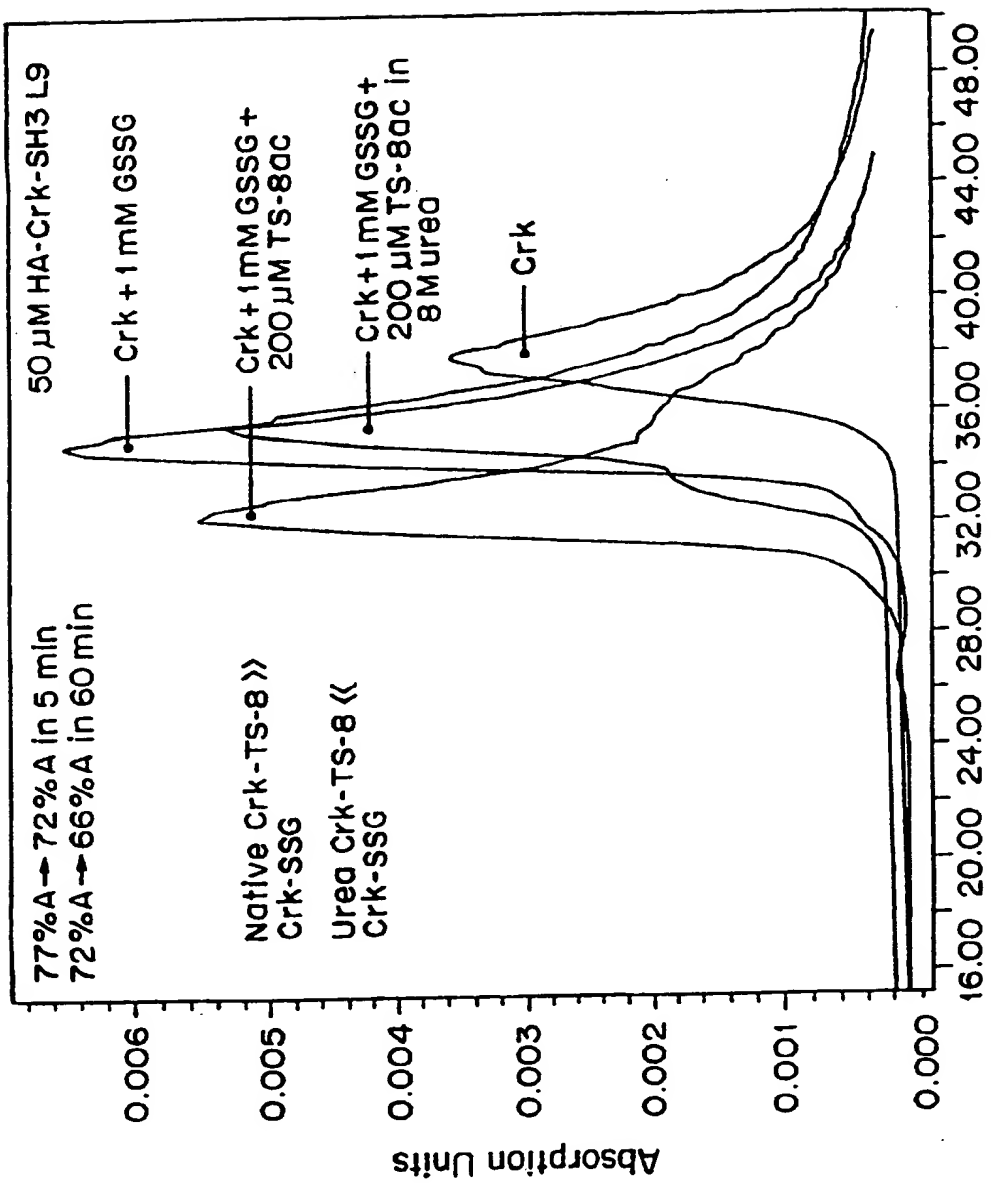
50 µM HA-Crk-SH3 L9
77%A→72%A in 5 min
72%A→66%A in 60 min
Crk + 1 mM GSSG
Crk + 1 mM GSSG + 200 µM TS-8ac
Crk + 1 mM GSSG + 200 µM TS-8ac in 8 M urea
Crk
Native Crk-TS-8 »
Crk-SSG
Urea Crk-TS-8 «
Crk-SSG
Absorption Units
0.000
0.001
0.002
0.003
0.004
0.005
0.006
16.00 20.00 24.00 28.00 32.00 36.00 40.00 44.00 48.00
Time (minutes)

FIG. 4

INTERNATIONAL SEARCH REPORT

Interi .onal Application No
PCT/US 97/16424

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/53 G01N33/543 G01N33/546

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	EP 0 801 307 A (CHUGAI BIOPHARMACEUTICALS INC) 15 October 1997 Whole document	1-83
E	WO 97 43302 A (HOLDINGBOLAGET VID GOETEBORGS ;AHLBERG PER (SE); BALTZER LARS (SE)) 20 November 1997 see claims 1-6 see page 5, line 7 - page 9, line 25	1-83
E	WO 97 35202 A (UNIV PRINCETON) 25 September 1997 see claims 1-19 see page 20, line 25 - page 21, line 19	1-83

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 February 1998	04/03/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Routledge, B

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter. .onal Application No
PCT/US 97/16424

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,A	WO 97 15831 A (SANGSTAT MEDICAL CORP ;LUSSOW ALEXANDER R (US); BUELOW ROLAND (US)) 1 May 1997 whole document	1-83
P,A	WO 97 00267 A (PENCE INC) 3 January 1997 Whole document	1-83
P,A	WO 96 41004 A (UNIV CALIFORNIA) 19 December 1996 Whole document	1-83

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter. .onal Application No
PCT/US 97/16424

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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WO 9735202 A	25-09-97	AU 2341397 A	10-10-97
WO 9715831 A	01-05-97	AU 7168996 A	15-05-97
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WO 9641004 A	19-12-96	NONE	